Letters to the Editor

Dear Sir:

A recent review by Dr. Mushak (*1*) discusses several of the procedures used to analyze for organic mercurials by gas chromatography. He points out that there are several problems associated with these procedures, including halide exchange and decomposition on-column, and difficulty in unambiguously identifying the "peak" as being due to the compound expected. We feel that the technical problems are more severe even than Dr. Mushak indicates, and would like to make available some of our observations.

We have experimented with the FDA procedure used by Drs. Kamps and Malone [derived from the various modifications of Westöö's method (*2–4*)], the method developed by T. W. Beak Consultants, Ltd. (*5*), and a procedure recommended to us by Dr. Mushak (*6*). Our instrumentation was basically the same as that described by Baughman et al. (*7*).

The major problems we encountered in methylmercury analysis seemed to fall under four categories: recovery, GLC liquid phases, injection techniques, and detection. Most of the published methods assume *either* that recovery of CH_3Hg from water is quantitatively comparable to recovery from a tissue homogenate, *or* that recovery of CH_3Hg from tissue homogenates is independent of the ratio of mercury to protein. In brief, both assumptions are incorrect. First, the recovery of CH_3Hg from water is commonly *less* than that from tissue homogenates, possibly because of the almost instantaneous loss of CH_3Hg from dilute aqueous solutions (*8*) in the absence of protecting agents.

We find the recovery of CH_3HgCl spikes from liver to fall on one recovery curve when the mercury concentration is >50 ppm, and another when the concentration is <50 ppm. The recovery from kidney was different from the recovery from liver at similar spiking levels. These variations were not seen with the FDA procedure (which uses large volumes, and huge excesses of extracting reagents), but this procedure assumes that recovery from water equals recovery from homogenates, which we could not confirm.

The FDA procedure uses phenyldiethanolamine succinate as a liquid phase. In our hands, water eluted from this packing at the same position as methylmercury chloride, the mercurial eluted before the electron capture detector had recovered from the solvent surge, and both effects made quantitation nearly impossible. The column packing recommended by T. W. Beak Consultants, Ltd., 7% Carbowax 20M on Varaport 30, had a very short usable lifetime (2–3 months), but worse, required that several injections of stock CH_3HgCl be made daily until the response reached a stable maximum. This resulted in the appearance of "ghost" peaks from blank injections. We tested two batches of Durapak Carbowax 400 on Porasil as recommended by Dr. Mushak. One batch performed as reported (*1*), while no methylmercury elution could be detected from the other. Only the former was specifically "low K."

Finally, since glass columns and on-column injection are necessary to avoid reaction of the mercurial with metal parts in the injection port, we had problems with decomposition of the liquid phases at the ends of the columns in contact with the injector and detector blocks. The addition of $HgCl_2$ to homogenates as recommended by Westöö gives a "ghost" peak in blanks if carbonization has occurred at the front of the GLC column.

We found it necessary to pull enough solvent (benzene or acetone) into the syringe ahead of the CH_3HgCl solutions to ensure

that all of the mercurial is rapidly flushed out of the needle. If this is not done, continued use of a particular syringe results in artifact peaks from methylmercury incapable of being washed out of the (stainless steel) needle but capable of being volatized out in the hot injection port.

The electron capture detector responds to the halide atom associated with methyl mercury. Although Baughman et al. (*7*) have demonstrated that methylmercury elutes as a mixture of chloride, bromide, and iodine if the three halides have ever had the opportunity to encounter the column packing, it would not appear to have been specifically pointed out that the detector will not be equally sensitive to the three halides. This phenomenon may account for much of the apparent day-to-day variations in detector sensitivity to methylmercury.

Those details of technique that have proven successful in overcoming the above problems in our laboratory are as follows. First, we use a column packing of 12% diethylene glycol succinate on HCl-washed Chromosorb W and never inject solutions containing halides other than chloride. We pack the front and back 4 in. of the column with 3% OV–1 on acid-washed Chromosorb W to eliminate decomposition in the heated blocks. We avoid extraction methods that require the use of iodide or bromide.

To cope with the recovery problems, we spike all samples with ethylmercury chloride before extracting, and then add 2,2′-dichlorobiphenyl to the final solution just prior to GLC. This procedure compensates for day-to-day variations in detector sensitivity, and gives a built-in measure of recovery of mercurial. Statistical analyses confirmed the considerable improvement in reproducibility and precision that these modifications made compared to use of recovery curves drawn for spiked (CH_3Hg) smples.

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